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Overnight Changes of Immune Parameters and Catecholamines Are Associated With Mood and Stress

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Abstract

Objectives—To test the hypothesis that a nocturnal decrease of secretion of inflammation markers and catecholamines would be associated with mood and stress variables even after controlling for objective sleep variables.

Methods—A total of 130 healthy volunteers participated in this study, spending 2 nights in the Gillin Laboratory of Sleep and Chronobiology at the University of California, San Diego, General Clinical Research Center. Blood samples were obtained before sleep (10:30 PM) and after awakening (6:30 AM) on the first day, and these samples were assayed for inflammatory biomarkers and catecholamines. On the second night, polysomnographic records were scored for objective sleep variables, e.g., total sleep time and wake after sleep onset. Self-rating scales for mood, stress, depression, and daily hassles were administered the second day.

Results—The nocturnal decrease in interleukin-6 was smaller in people who reported more negative mood or fatigue and greater in those who reported more uplift events (e.g., with Profile of Mood States fatigue $r_p = -.25$ to $-.30$). People with high stress or high depression levels had smaller nocturnal decreases of epinephrine. That relationship was even stronger when partial correlations were used to control for morning level and sleep variables. The associations between nocturnal changes of C-reactive protein, soluble tumor necrosis factor-receptor I, and norepinephrine with psychological states were nonremarkable.

Conclusions—The analyses of nocturnal change scores (difference scores) add substantial information compared with the traditional analyses of morning levels of immune variables and catecholamines alone. Subjective well-being is significantly associated with a greater nocturnal decrease of interleukin-6 and epinephrine. More research on nocturnal adaptation processes is warranted.

Keywords

sleep; stress; mood; cytokines; sympathetic nervous system; chronobiology

INTRODUCTION

Day to night variation in physiological processes is extensive. The amplitude of these circadian rhythms is considered to indicate health, and long-term disruption of these rhythms

correlates with disease states (1). The origins of such variation may stem from so-called internal body clocks that are even demonstrated on a single cell level (1), or from changes in activity, such as bed rest. Because, in general, nocturnal rest reduces physiological arousal after daytime stress experiences, one might expect that hormones sensitive to stress would be lower first thing in the morning hours compared with the previous evening hours. One way of examining this topic is to contrast the change in hormone level from evening to morning. A large delta (Δ) (evening value minus morning value) would indicate high nocturnal decrease of its concentration. Although the meaning of humoral nocturnal declines is poorly understood, more research has been published dealing with nocturnal declines in blood pressure, where the degree of nocturnal dipping seems to be attenuated in people with more depressive symptoms or who experience more stressful daytime events (2,3). Attenuated nocturnal blood pressure dipping and heart rate dipping are associated with increased cardiovascular morbidity and mortality (4–6).

Similarly, the diurnal changes in cortisol, for example, are well known, and numerous associations have been reported between elevated early morning cortisol levels and depressive symptoms, stressful life events, and social adversity (7–11). Curiously, psychosocial determinants of nocturnal recovery of immune variables and indices of sympathetic nervous system activity have not typically been investigated. Therefore, we wanted to analyze whether nocturnal changes of immune and sympathetic nervous system parameters were associated with psychosocial variables reflecting well-being or adversity.

Overactivation of the immune system and increased secretion of immune parameters (e.g., interleukin [IL]-6, IL-1, tumor necrosis factor [TNF]- α) is associated with negative mood (12–14). Several proinflammatory cytokines show increased concentrations in depression (15,16). Increased morning cortisol and serum IL-6 levels predict the development of posttraumatic stress disorder in children and adolescents (17). If morning IL-6 concentrations are lower in healthy individuals than in patients with posttraumatic stress disorder, this could partly reflect different overnight decreases of IL-6. Such observations could imply that difference scores of bedtime versus next morning concentrations of immune parameters should correlate with aspects of adversity or well-being.

Sleep itself can be considered as a potential link between psychological well-being and nocturnal immune changes. For instance, increased morning IL-6 levels have been associated with sleeping problems, such as greater wake after sleep onset (WASO) (18). Burgos et al. (19) investigated 11 patients with primary insomnia and found higher nocturnal IL-6 excretion in the insomniacs as compared with participants without sleeping problems. Perry et al. (20) confirmed diurnal variations of cytokines and postulated a similar overnight variation of IL-6 compared with cortisol with a rise in the early morning hours. Moreover, an association of nocturnal wake time with 24-hour IL-6 concentrations was also confirmed (21). The same group reported nocturnal variations not only for IL-6 but also for TNF- α (22). In addition, daytime napping after a night of sleep loss results in decreases in cortisol and IL-6 (23). If sleeping patterns of patients with obstructive sleep apnea syndrome are normalized using continuous positive airway pressure, serum IL-6 levels are significantly reduced (24). All of these results suggest that restful sleep might be associated with a reduction of proinflammatory cytokines. Because sleep disturbance and negative mood can affect one another, it remains unclear whether negative mood is directly related with nocturnal immune changes, or whether such an association is explained by disturbed sleep parameters. With polysomnographic (PSG) monitoring, it is possible to determine if the Δ hormone value is more closely associated with the stressful daytime experiences or with disruptions of sleep itself.

Whereas several studies have investigated either evening or morning levels of immune variables, the overnight change in levels has seldom been investigated. We postulate that it is not only the level per se but the nocturnal decrease from evening to morning scores that is associated with sleep quality and psychological states. We analyzed covariates of nocturnal changes of immune variables and catecholamines, and we hypothesized that nocturnal reductions of the concentration of these biological parameters would be associated with psychological states. To analyze whether nocturnal difference scores add information to the analysis of morning levels, these associations were controlled for morning levels of the corresponding biological variables. Moreover, potential associations of nocturnal decrease of immune parameters with psychological states were controlled for objective sleep variables to analyze whether they could be explained by sleep problems that frequently coexist with depressed mood.

METHODS

As part of a larger study on health of African Americans and white Americans, this study examined 130 healthy controls, who were recruited between 2006 and 2009 using advertisements and announcements. Mean age was 35 years, and 43% were female. The sample was roughly evenly divided between white and African Americans (Table 1). Paid volunteers stayed in the Gillin Laboratory for Sleep and Chronobiology at the University of California, San Diego General Clinical Research Center for 2 nights for sleep monitoring, psychological testing, and blood sampling. Exclusion criteria were: current heart disease other than hypertension, serious pulmonary disease, history of psychosis, current alcohol or drug abuse, moderate or heavy smoking (>10 cigarettes/day), increased caffeine intake (>600 mg/day), hormone therapies including contraceptives, pregnancy, and medication use other than antihypertensives. If participants were taking antihypertensive medication, they were tapered off with the permission of their prescribing physician and underwent a 3-week washout period before study participation. The study was approved by the University of California, San Diego Institutional Review Board.

Procedure—After prescreening, participants were invited to come to the Gillin Laboratory for Sleep and Chronobiology at the University of California, San Diego General Clinical Research Center at 8:30 PM. They were hooked-up for an acclimation night of sleep monitoring, and an evening blood sample was taken at 10:30 PM before lights were turned off for sleeping. The morning blood sample was taken after waking the subject at 6:30 AM the next day independently of sleep stage. Both blood samples were taken from subjects while they were recumbent in bed. During the next night, sleep was monitored with PSG. Time with lights off was identical between the first and second nights. The sleep rooms were 180 ft (2) sound-shielded rooms with a wall port for equipment cables. Sleep data acquisition was performed with PSG system (A10, Embla Systems Inc., Broomfield, Colorado) and included electroencephalography (C4/C3), electrooculography, chin electromyography, leg electromyography, airflow with an oronasal thermal sensor, airflow with an air pressure transducer, snore events with a piezo snore sensor, respiratory effort with piezo thoracic and abdominal belts, oxygen saturation with a pulse oximeter sensor, and body position with a position indicator. Recordings were scored by a trained sleep technician according to criteria from Rechtschaffen and Kales (25). From the PSG, we computed total sleep time (TST), WASO, and the Apnea-Hypopnea Index (AHI). TST was calculated as the total minutes from sleep onset to wake-up and includes time spent in Stages 1 through 4 of sleep and rapid eye movement. WASO was considered to be the number of minutes spent awake after initiating sleep. Apneas were defined as decrements in airflow of $\geq 90\%$ from baseline for ≥ 10 seconds. Hypopneas were defined as decrements in airflow of $\geq 50\%$ but $< 90\%$ from baseline for ≥ 10 seconds. Significant oxyhemoglobin desaturations

were defined as transient drops in oxyhemoglobin saturation by $\geq 3\%$ from baseline. The total number of apneas and hypopneas per hour of sleep were calculated to yield the AHI. Further sleep variables are presented in Table 1.

There is a trade-off between response burden on subjects and optimal data integrity for testing hypotheses. We used a two-night protocol to be able to recruit a broad cross-section of subjects who were working. A longer protocol would have increased response burden. We separated the sleep recording night from the blood sampling night because of the effect of blood sampling on sleep parameters. The second night followed immediately to the first night. Activities between the two nights were ad libitum to allow the continuation of everyday activities.

Participants were provided with questionnaires on admission and required to complete them before discharge. The following questionnaires were used: The Profile of Mood States (POMS) (26) to assess tension, depressed mood, anger, vigor, fatigue, confusion, as well as a total mood disturbance score; the Center for Epidemiological Studies Depression Scale (27); the Combined Hassles and Uplift Scale (28) to assess hassle frequency and severity, as well as uplifts frequency and severity; the Perceived Stress Scale (PSS10) revealing a general index for perceived stress (29); the total fatigue score derived from the short form of the Multidimensional Fatigue Symptom Inventory (MFSI) (30); and the global score of the Pittsburgh Sleep Quality Index (PSQI) (31).

Blood samples were obtained through an intravenous catheter to remove effects of pain caused by the needle stick. The intravenous catheter was inserted after admission around 4:30 PM. The bedtime draw was done around 10:30 PM and the waking draw was done around 7:30 AM the next morning. Participants were lying in their bed to avoid effects of movements or physical activities on immune biomarker levels and catecholamines. Plasma was stored at -80°C until assay. C-reactive protein (CRP) was assayed using the high sensitivity Denka-Seiken method (32). Intra-assay and interassay coefficients of variation were $<3\%$; assay sensitivity was <0.05 mg/L. IL-6 and soluble TNF-receptor I (sTNF-RI) were determined by commercial ELISA (Quantikine, R&D Systems, Minneapolis, Minnesota). Intra-assay and interassay coefficients of variation were $<5\%$; assay sensitivity was <0.72 pg/mL and <0.61 pg/mL for IL-6 and sTNF-RI, respectively. Catecholamines were determined using a COMT-based radioenzymatic assay (33). Intra-assay and interassay coefficients of variation were $<7\%$ and $<11\%$, respectively.

All assays were run using samples that had not been previously freeze-thawed. To minimize intra-assay variance, both samples from each participant were analyzed within the same assay run.

Statistics

As a first step, the mean and standard deviation values of all variables were computed. All biological parameters of >3 standard deviation from the mean were considered to be outliers and excluded from the corresponding analysis. This was the case for $\leq 5\%$. All participants with CRP scores of >10 mg/L were also excluded because of potentially ongoing infectious diseases. For all biological parameters, difference scores of the evening minus morning concentrations were computed. Positive values indicate a nocturnal decrease of the variable. Because parametrical analyses in sample sizes >100 are quite robust even if normal distribution is not fully achieved, we did not log-transform these difference scores. Afterward, these biological change scores were correlated with the psychological variables.

If significant associations were found between biological change scores and psychological variables, we examined whether these variables shared variance that was not already

included in associations of psychological variables with the biological baseline measure (morning concentrations). Therefore, these correlations were reanalyzed computing partial correlations correcting for morning levels of the biological variables. If these partial correlations were still significant, it implied that the nocturnal change scores included additional information compared with the morning levels of these variables.

Significant associations between psychological variables and biological change scores were corrected for objective sleep variables, again computing partial correlations and correcting for the influence of TST, WASO, and AHI. If the correlations still remained significant, it implied a direct association of psychological states with nocturnal biological change scores that was not mediated by objective sleep quality. Finally, if more than two psychological variables correlated with one biological change score, linear regression analysis was computed to control for interdependency of psychological variables. To visualize findings that are stable even after extracting the effect of sleep and morning variables, we occasionally dichotomized psychological variables (e.g., fatigue) and reported full data for biological nighttime and morning scores.

Adjusting for Multiple Testing

Interpreting significance levels from a large correlation matrix requires adjusting for multiple testing to correct for significant coefficients that occur just by chance. Because of the known conservative bias of the Bonferroni procedure (34), we used a modification of the Holm's method (35) to correct for multiple testing in correlation matrices. Significant results are organized in increasing order according to their significance levels (or correlation coefficients). Then, it is defined how many significant correlations can be expected by chance due to multiple testing with α 5%, and the list of significant correlations is reduced according to this number, starting with the lowest coefficients that were significant on an individual level. This is still a conservative approach, as it assumes that significance levels of all coefficients are equal to 5%, whereas most are <5%. We report results with and without adjustment for multiple testing. Correlation coefficients with control variables (e.g., sleep parameters) were not adjusted for multiple testing, as it is more conservative not to exclude control variables prematurely.

RESULTS

Complete Data Sets

Due to the multiday assessments and occasional technical problems, missing values occurred. However, we obtained psychological tests on >90% of participants, sleep variables for 124 of 130 participants, IL-6 for 112 participants, CRP for 122, and sTNF-R1 for 105 of the 130 participants. Catecholamine data were received for 84 (Δ epinephrine) and 89 (Δ norepinephrine) participants (Table 1).

Role of Sleep Variables

The objective PSG sleep variables TST, WASO, and AHI were not significantly associated with any of the biological change scores. All correlations of nocturnal changes of catecholamine with objective sleep variables were below $r < .09$ and nonsignificant. None of the overnight immune changes correlated significantly with objective sleep variables, with correlation coefficients ranging from .04 to .16. Analyzing associations with evening and morning concentrations separately, the only significant correlations were found for CRP, which was related to the AHI index (CRP 22:30 PM: $r = .24$; $p < .01$; CRP 6:30 AM: $r = .18$; $p < .05$).

For exploratory reasons, we also assessed subjective sleep duration (according to PSQI subscale) to extend potential findings on objective sleep variables. This variable showed a significant positive association with a nocturnal decrease of CRP ($r = .22, p < .05$) and a negative association with a nocturnal decrease of norepinephrine ($r = -.26; p < .05$).

The POMS fatigue score and the MFSI fatigue score were highly correlated ($r = .75; p < .001$). They were not significantly associated with objective sleep variables (all $r = -.10$).

Psychological Correlates of Nocturnal IL-6 Decrease

Table 2. shows that the nocturnal decrease of IL-6 was related to a variety of psychological dimensions. Seven of the 15 correlations in Table 2 were significant. Modified Holm adjustment for multiple testing denies two of them. The nocturnal decrease of IL-6 was related to positive daily events (uplift frequency, uplift severity), and negatively associated with feelings of tension and fatigue. Both fatigue scales (MFSI total fatigue score and POMS fatigue score) indicated less nocturnal decrease of IL-6 if participants reported more fatigue.

It could be hypothesized that the nocturnal decrease of IL-6 was a mere reflection of the general level of IL-6 concentrations, with elevated levels of IL-6 being related to more pronounced nocturnal decrease. Table 3 (third column) shows the partial correlations of all significant associations of Δ IL-6 with psychological variables when these correlation coefficients were controlled for IL-6 morning scores. Three of the seven significant associations remained significant, implying that additional information was included in Δ IL-6 that was not provided by IL-6 morning levels. Two of the seven significant associations were slightly reduced but remained in the range of $0.05 < p < .10$. Only two of the seven significant associations between Δ IL-6 and psychological variables were substantially explained by morning levels of IL-6. Vice versa, if evening IL-6 scores instead of morning IL-6 scores were used as a control variable in partial correlation analyses, five of the seven associations between nocturnal IL-6 decrease and psychological variables remained significant or showed a trend to significance (at least $p < .07$). Again, this confirms that nocturnal IL-6 decrease scores include additional information compared with general concentration scores.

Although objective sleep variables did not show significant associations with nocturnal IL-6 changes, it could still be possible that they contributed to these associations. Therefore, we reanalyzed the associations of Δ IL-6 with psychological variables controlling for the three objective sleep variables. The last column of Table 3 shows that nearly all associations between Δ IL-6 with psychological variables remained or even increased after controlling for objective sleep variables.

Because seven psychological variables correlated with Δ IL-6, we also computed linear stepwise regression analysis including Δ IL-6 as criterion, and all seven psychological variables showing significant associations with Δ IL-6 as potential predictor variables. Two psychological variables remained in the regression model, which explained a significant amount of variance ($F = 7.8, df 2,95; p < .001$). The POMS Fatigue Scale covered the more negative emotions (standardized beta = $-0.32; p < .001$), whereas the uplift frequency added additional information to the prediction of Δ IL-6 (standardized beta + $0.22; p < .05$).

To visualize these associations with an example, we dichotomized the variable fatigue using split-half (POMS fatigue score of >3 equals "high fatigue") and computed a repeated-measure analysis of variance with time point as repeated measure and fatigue as group factor. Neither fatigue nor time point revealed significant main effects on IL-6, but the interaction was significant ($F = 7.5; df 1,110; p < .008$). This interaction is shown in Figure

1, and it further underlines that nocturnal change scores can reveal information that is not included in the evening or morning level scores.

Psychological Correlates of Nocturnal Decrease of Other Biological Variables

The nocturnal CRP changes were associated with only one of the psychological variables, namely, the total stress score PSS10 ($r = .20$; $p < .05$), and this association was questioned by adjustment for multiple testing. If this association was controlled for morning scores of CRP, it declined to $r = .15$ (NS). The nocturnal changes of sTNF-R1 were associated with mood variables, namely, POMS fatigue (-0.22 ; $p < .05$), whereas the associations with POMS anger (-0.19) and the MFSI Fatigue Scale (-0.16) failed to reach significance. Moreover, the significant association of sTNF-R1 with POMS fatigue is questioned after adjustment for multiple testing.

The nocturnal epinephrine decrease was significantly associated with depression and stress (Center for Epidemiological Studies Depression Scale, $r = -.29$; PSS10, $-.24$; $p < .05$). It also showed relevant associations with other mood scales (POMS tension, -0.20 ; POMS vigor, $+0.21$; total fatigue score, -0.23 ; all NS), but due to the smaller sample size of the catecholamines, these associations failed to reach significance levels. Interestingly, these associations remained completely unchanged after controlling for morning scores of epinephrine and for objective sleep variables (Table 3). Therefore, the nocturnal decrease of epinephrine and its association with depression and subjective stress were not explained by the general level of catecholamines. This result again underlines the relevance of analyzing nocturnal change scores in addition to general level scores. The nocturnal changes of norepinephrine were not associated with any of the psychological variables included in this study. Only the subjective sleep duration, which was included in these analyses due to exploratory reasons (compared with objective sleep duration), showed some association with this variable.

DISCUSSION

Our results showed that the nocturnal change scores for immune variables and catecholamines provide additional information that is not provided by analyzing morning levels of these biological variables. The nocturnal decrease of IL-6 showed several associations with psychological variables, such as mood, fatigue, and the frequency of uplifting events. However, the direction of causality remains open, as all data were based on correlations. That is, it is possible that an insufficient nocturnal readjustment of biological systems induces or facilitates the development of negative mood and feelings of fatigue. Alternatively, uplifting events and low scores of negative mood could facilitate nocturnal IL-6 decreases, whereas feelings of fatigue, being exhausted, or other mood variables could hinder the nocturnal IL-6 decrease. The negative associations between stress level and depressive symptoms with nocturnal decrease of epinephrine point to the same direction as the IL-6 results. Depressive symptoms and the total stress level seem to hinder a nocturnal decrease of epinephrine or are amplified by low overnight recovery of the adrenergic systems.

Nocturnal variations of human biological systems play a major role for readjustment and for the preparation of daily adaptation processes. Typically, most (but not all) biological systems reduce their activity during the night hours. Heart rate and blood pressure decrease, usually in association with the reduction of secretion of major stress hormones, and it has been shown that nocturnal change scores of cardiovascular activity (“nocturnal dipping”) (2,3) add information to the general levels of heart rates and blood pressure. The secretion of catecholamines decreases overnight (36). Cortisol as a prominent representative of the hypothalamic-pituitary-adrenal axis shows strong diurnal and nocturnal variations, with a

reincrease of cortisol secretion in the late night/early morning hours (20). Generally, high amplitude rhythms are regarded as “healthy,” whereas flattened circadian neuroendocrine rhythms have been reported in the elderly and in people with depression (37). Poor sleep is also considered to cause a disruption of circadian rhythms (11).

Despite earlier studies and overviews, information on diurnal and nocturnal changes for cytokines and cytokine-specific receptors is still limited (38–42). Although the hypothalamic-pituitary-adrenal axis obviously influences cytokines (43), there is also direct evidence for nocturnal variations of immune parameters. The concentration of CD4-helper cells and CD8-suppressor cells is lowest in the early morning hours (44). Sleep seems to increase the number of IL-12-producing monocytes but decreases the number of IL-10-producing monocytes (45), hereby modulating the Type 1/Type 2 helper T cells (Th1/Th2) relation of the immune response.

Impaired night time sleep has been found to be associated with elevated plasma IL-6 levels (21,46). Hong et al. (18) showed that morning IL-6 levels were positively correlated with WASO and negatively correlated with sleep efficiency, a result we did not replicate for nocturnal change scores. Nocturnal IL-6 excretion was increased in patients with primary insomnia (19), which could be also related to insomnia-associated states of negative mood. This effect seems to be most pronounced around midnight (22). Therefore, it has been postulated that the circadian secretion of IL-6 correlates with sleepiness (46,47). This assumption was confirmed by our results, showing that the nocturnal IL-6 decrease was negatively associated with feelings of fatigue, a result confirmed by both scales assessing fatigue. On the other hand, positive daytime experiences (uplift frequency) were associated with a more pronounced decrease of nocturnal IL-6 concentrations. However, the cited studies assessing diurnal and nocturnal variations of IL-6, as well as our IL-6 results, should be interpreted with caution. Diurnal variations are confounded by blood drawing procedures (48,49), and differences in blood drawing techniques between studies could have led to inconclusive results for IL-6. The nocturnal changes of CRP were only weakly related to stress and subjective sleep duration, and after adjustment for multiple testing, the correlation with stress dropped under the significance level. It may be that the amplitude change in CRP over 24 hours was too small to be detected in this sample, or the timing of such changed levels departed from our sampling at 10:30 PM and 6:30 AM. Similarly, for sTNF-R1, diurnal and nocturnal variations were unclear. In our sample, the mean scores even increased from bed time to awake. Comparable results indicating a slight increase of soluble TNF receptors have been reported elsewhere (42). Therefore, the reported association of nocturnal sTNF-R1 changes with fatigue should not be overinterpreted, especially because it was questioned after α adjustment.

Catecholamines are not only relevant for their role in the sympathetic nervous system, but are prominently influenced by emotional factors. Brummett et al. (50) were able to show that positive affect was inversely related to the average level of norepinephrine. In our data, the nocturnal decrease of epinephrine was associated with low levels of daily stress and low depression scores. Interestingly, this association was even stronger after controlling for morning levels of epinephrine and after controlling for objective sleep variables. Again, this underlines that the nocturnal change scores included information that went beyond the mean level scores, and that these nocturnal changes were linked to psychological well-being. Moreover, in light of the association of IL-6 concentrations with blood pressure (51), complex interactions of catecholaminergic systems and immunity should be considered.

Our results also provide some information on the concept of fatigue. The similarity of results for different fatigue scales confirms the homogeneity of the construct. On the other hand, fatigue and sleepiness seem to relate to different underlying concepts, with fatigue being less

associated with nonrestorative sleep (52). This is confirmed by lacking associations of fatigue with objective sleep parameters in our study, whereas fatigue was closer related with variables of emotional well-being.

Finally, we summarize some weaknesses and strengths of this study. Due to multiple testing, some of the reported associations could be expected just by chance. Although we used an adjustment procedure for multiple testing, some of the reported correlations could still be artifacts. We favor the view that the correlation coefficients should only be interpreted if they are either confirmed by other studies, or are part of a group of correlation coefficients indicating a comparable direction (as for example for IL-6). Another limitation was the assessment of nocturnal changes with only one evening assessment (10:30 PM), and one assessment after awakening (6:30 AM), but without continuous assessments over 24 hours. Comparable to cortisol, some of the immune variables and catecholamines might show a rise in the early morning hours which reduces or reverses the nocturnal decrease of the corresponding system (53). Some researchers used more assessment points and computed cosinor modulation. However, these models are frequently based on small sample sizes, whereas our study was based on a large sample size with standardized assessment procedures, including objective sleep measures.

Further limitations resulted from the study design. We tried to disentangle the effects of blood sampling and polysomnographic sleep assessments, which resulted in a two-night design, but the design cannot exclude the possibility of a “first-night effect” influencing the blood sampling. To fully disentangle this would have required a lengthier hospitalization which, in turn, would have been more burdensome. The associations between immune parameters and sleep variables could be weakened by the fact that these measures were separated by one day, but the fact remains that associations were found despite the time lag between assessments. Finally, it might have been advantageous to have had the self-rating scales completed at one consistent time of day, but most of these scales ask the respondent to report on activities over the course of several days (e.g., PSQI sleep quality asks for the last 7 days). Thus, we doubt that time of day of scale administration was a major confound.

To our knowledge, this is one of the first studies demonstrating that nocturnal change scores of immune parameters and catecholamines contain additional information that is not covered by the general morning baseline level of these variables. Therefore, continuing the research of nocturnal change scores as a potential index of healthy adaptation processes and their association with psychosocial factors is warranted.

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Glossary

Δ	delta, difference score
AHI	Apnea-Hypopnea Index
CRP	C-reactive protein
IL	interleukin
MFSI	Multidimensional Fatigue Symptom Inventory
POMS	Profile of Mood States
PSG	polysomnography

PSQI	Pittsburgh Sleep Quality Index
PSS-10	Perceived Stress Scale
sTNF-RI	soluble tumor necrosis factor-receptor I
TNF	tumor necrosis factor
TST	total sleep time
WASO	wake after sleep onset

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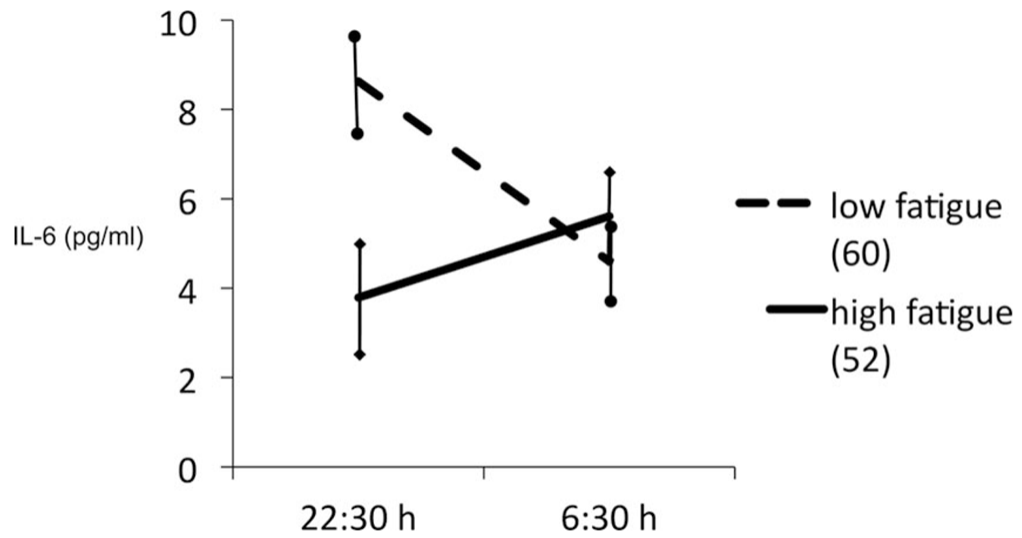


Figure 1. Bedtime and awake concentrations of interleukin (*IL*)-6 in people with high and low fatigue scores. Observed means; bars indicate standard error of the mean values; groups according to split-half procedure of Profile of Mood States fatigue score. Analysis of variance interaction term $F = 7.5$; $df 1,110$; $p < .008$.

TABLE 1

Sociodemographic and Clinical Sample Characteristics

	Valid <i>n</i>	<i>n</i> (%) or Mean	SD
% female	130	56 (43%)	
Hispanics	130	8 (6%)	
African Americans	130	60 (46%)	
White Americans	130	62 (48%)	
Employed	130	120 (92%)	
Age	130	34.9	9.6
BMI (self-report)	129	25.6	3.87
CHUS Hassle Frequency	120	25.0	11.12
CHUS Hassle Severity	119	1.50	.37
CHUS Uplift Frequency	118	33.2	10.30
CHUS Uplift Severity	117	1.86	.39
PSS10 Total Score	122	13.1	6.5
MFSIsf Total	118	3.35	16.4
POMS Tension	121	4.59	4.5
POMS Depression	121	3.94	5.5
POMS Anger	120	3.73	4.6
POMS Vigor	121	12.35	5.3
POMS Fatigue	121	4.56	4.5
POMS Confusion	121	3.39	3.8
POMS Total	120	7.84	22.0
CES-D Depression Score	117	11.24	8.98
PSQI Sleep Duration	120	.72	.93
PSQI Global Score	120	5.30	3.15
Total sleep time (min)	124	394.1	54.43
Wake after sleep onset (min)	124	24.27	22.61
AHI	124	10.00	20.44
REM Sleep (min)	124	87.3	28.1
Stage 1 sleep (min)	124	24.2	17.1
Stage 2 sleep (min)	124	201.9	45.5
Stage 3 sleep (min)	124	30.3	14.8
Stage 4 sleep (min)	124	50.4	34.8
CRP 10:30 PM Bedtime (mg/L)	122	1.03	1.32
CRP 6:30 AM Awake (mg/L)	125	1.07	1.41
ΔCRP (mg/L)	122	.048	.27
IL-6 10:30 PM Bedtime (pg/mL)	118	6.40	9.45
IL-6 6:30 AM Awake (pg/mL)	112	5.07	7.31
Δ IL-6 (pg/mL)	112	1.31	11.65
sTNF-RI 10:30 PM Bedtime (pg/mL)	105	939.8	284.3
sTNF-RI 6:30 AM Awake (pg/mL)	107	1026.0	346.5

	Valid <i>n</i>	<i>n</i> (%) or Mean	SD
Δ sTNF-RI (pg/mL)	105	-89.36	228.03
Epinephrine 10:30 PM Bedtime (pg/mL)	91	38.09	41.8
Epinephrine 6:30 AM Awake (pg/mL)	85	25.43	13.1
Δ Epinephrine (pg/mL)	84	13.47	43.2
Norepinephrine 10:30 PM Bedtime (pg/mL)	95	444.5	216.8
Norepinephrine 6:30 AM Awake (pg/mL)	89	369.5	144.2
Δ Norepinephrine (pg/mL)	89	76.39	176.7

SD = standard deviation; BMI = body mass index; CHUS = Combined Hassles and Uplift Scale; PSS10 = Perceived Stress Scale; MFS1sf = Multidimensional Fatigue Symptom Inventory-Short Form; POMS = Profile of Mood States; CES-D = Center for Epidemiological Studies Depression Scale; PSQI = Pittsburgh Sleep Quality Index; AHI = Apnea-Hypopnea Index; REM = rapid eye movement; CRP = C-reactive protein; IL = interleukin; sTNF-RI = soluble TNF-receptor I.

TABLE 2

Associations of Biological Overnight Changes With Psychological Variables

Subjective Variables	Δ IL-6	Δ CRP	Δ sTNF-R1	Δ Epinephrine	Δ Norepinephrine
CHUS Hassle Frequency	0.140	-0.079	0.032	-0.167	0.073
CHUS Hassle Severity	-0.055	-0.025	0.066	-0.109	-0.003
CHUS Uplift Frequency	0.223*	-0.082	0.116	-0.026	-0.045
CHUS Uplift Severity	(0.212*)	-0.130	-0.058	-0.026	-0.153
PSS10 Stress Score	-0.077	(0.198*)	-0.035	-0.241*	0.065
MFSIsf Fatigue Score	-0.268**	-0.062	-0.159	-0.226	0.022
POMS Tension	-0.220*	-0.003	-0.112	-0.203	-0.142
POMS Depression	-0.122	0.025	0.009	-0.103	0.028
POMS Anger	-0.226*	0.043	-0.190	-0.013	-0.058
POMS Vigor	0.033	-0.067	0.080	0.206	-0.177
POMS Fatigue	-0.255**	-0.029	(-0.218*)	-0.115	-0.103
POMS Confusion	-0.142	-0.001	-0.051	-0.189	-0.054
POMS Total	(-0.208*)	0.025	-0.132	-0.171	-0.023
CES-D Depression Score	-0.002	-0.025	-0.004	-0.285*	-0.082
PSQI Global Score	-0.059	0.120	-0.017	-0.145	-0.118

Numbers in parentheses are correlation coefficients that should not be interpreted as significant after applying the modified Holm's method for multiple testing.

Text in bold represents significant bivariate correlation coefficients.

* $p < .05$;

** $p < .01$.

IL = interleukin; CRP = C-reactive protein; sTNF-R1 = soluble TNF-receptor I; CHUS = Combined Hassles and Uplift Scale; PSS10 = Perceived Stress Scale; MFSIsf = Multidimensional Fatigue Symptom Inventory-Short Form; POMS = Profile of Mood States; CES-D = Center for Epidemiological Studies Depression Scale; PSQI = Pittsburgh Sleep Quality Index.

TABLE 3

Partial Correlations After Controlling for Morning Scores and for Sleep Variables

	<i>r</i> Without Controlling for Other Influences	<i>r_p</i> After Controlling for Morning Scores	<i>r_p</i> After Controlling for Objective Sleep Variables
7Δ IL-6 with			
CHUS Uplift Frequency	.22*	.21*	.22*
CHUS Uplift Severity	(.21*)	.15	.19 [†]
MFSIsf Fatigue Score	-.27**	-.26**	-.30**
POMS Tension	-.22*	-.19 [†]	-.25*
POMS Anger	-.23*	-.15	-.28**
POMS Fatigue	-.26**	-.25*	-.30**
POMS Total	(-.21*)	-.19 [†]	-.26*
Δ CRP with			
PSS10 Stress score	(.20*)	.15	.19*
PSQI subj. sleep duration	.22*	.16	.20*
ΔsTNF-R1 with			
POMS Fatigue	(-.22*)	-.19 [†]	-.22*
Δ Epinephrine with			
PSS10 Stress score	-.24*	-.30*	-.31**
CES-D Depression Scores	-.29*	-.28*	-.31**
Δ Norepinephrine with			
PSQI subj. sleep duration (variable included only for exploratory reasons)	-.25*	-.26*	-.25*

Numbers in parentheses are correlation coefficients that should not be interpreted as significant after applying the modified Holm's method for multiple testing.

* $p < .05$;

** $p < .01$;

[†] $.05 < p < .10$.

IL = interleukin; CHUS = Combined Hassles and Uplift Scale; MFSIsf = Multidimensional Fatigue Symptom Inventory-Short Form; POMS = Profile of Mood States; CRP = C-reactive protein; PSS10 = Perceived Stress Scale; sTNF-RI = soluble TNF-receptor I; CES-D = Center for Epidemiological Studies Depression Scale; PSQI = Pittsburgh Sleep Quality Index.